REMARKS

The specification is herein amended at page 9 to correct a clerical or translation error.

Claims 8 and 12 were withdrawn by the Examiner as drawn to a non-elected invention. Claims 1-6, 9-11, 13-16, 18-20 and 22 are amended and new claim 25 is added.

Therefore, claims 1-7, 9-11 and 13-25 are currently pending.

The Invention

The present invention provides compositions of micelles or micro-aggregates that include a lipopeptide that contains at least one CTL antigenic determinant and at least one lipid unit as well as another lipopeptide that contains at least one helper T antigenic determinant and at least one lipid unit. These micelles or micro-aggregates are capable of simultaneously presenting the lipopeptides to the APC and thereby inducing an immune response.

Also provided are methods of preparing these micelles or micro-aggregates by dispersing each of the constituent lipopeptides in a solution of concentrated acetic acid and mixing to obtain the micelles or micro-aggregates.

Further, the invention provides vaccines and pharmaceutical compositions that include these micelles or micro-aggregates that are useful for inducing an immune response.

The Office Action of July 5, 2001

In the Office Action of July 5, 2001 the Examiner withdrew claims 8 and 12 from consideration as pertaining to a non-elected invention.

Rejections under 35 U.S.C. §112, second paragraph

In the Office Action the Examiner rejected claims 1-7, 9-11, 13-24 as being allegedly indefinite.

Claim 1: According to the Examiner, claim 1 was unclear as to whether or not both lipopeptides are contained in a micelle or if each micelle contains one of the lipopeptides, which are brought together in a mixture.

In response, Applicants have amended claim 1 to recite "comprising micelles or micro-aggregates, wherein each micelle or micro-aggregate comprises a first lipopeptide..., and a second lipopeptide..."

By this amendment Applicants claim compositions containing micelles or micro-aggregates that each contain both lipopeptides. Applicants therefore assert it is clear that there can be no confusion as to whether the lipopeptides are in different micelles or micro-aggregates or are mixed within each micelle or micro-aggregate.

Claim 13: According to the Examiner, the recitation of inducing a "specific immune response" with the micelles [or micro-aggregates] in claim 13 was unclear. The Examiner asks whether the immune response is the CTL response or the T helper response. Further the Examiner inquires, whether the claim is drawn to a specific immune response against a specific antigen.

In response, Applicants have deleted the term "specific" from claim 13. Therefore, Applicants submit that this rejection of claim 13 is no longer valid and should be withdrawn.

Claim 18: The Examiner states that the recitation of NMR "controlling" the dispersal of lipoproteins in claim 18 is unclear in that NMR is not conventionally known to facilitate dispersing or mixing.

In response Applicants have amended claim 18 to recite dispersing of the lipopeptides "confirmed" by NMR. This amendment constitutes a more accurate translation of the English specification from the original French priority document.

Rejections under 35 U.S.C. §112, first paragraph

Claims 13, 14, 16 and 20: At page 3 of the Office Action the Examiner rejected claims 13, 14, 16 and 20 for alleged lack of enablement. According to the Examiner, in order to meet the legal requirements for patentability, claims to a treatment method "must be effective in

treating or preventing the indicated disease conditions." Further, the Examiner goes on to state that "an applicant can be required to provide evidence to substantiate the assertions of effectiveness." and that there is no working example of the treatment method demonstrating a prophylactic effect of the composition in an individual or animal model against HIV. The Examiner acknowledged the examples of antibody responses and *in vitro* PBMC proliferation. According to the Examiner, the specification does not disclose data that a proliferative response in CD4+ T cells would be a beneficial treatment in an individual with HIV.

In summary, according to the Examiner, there is insufficient evidence to convince one of skill in the medical arts of the effectiveness of the composition a vaccine and that undue experimentation would be required to meet the enablement standard for the invention as claimed.

In response, Applicants provide two reports from scientific journals co-authored by the present inventors that demonstrate effectiveness of the claimed vaccine compositions in generating CTL as well as B-cell and T-cell responses *in vivo*. These data unequivocally demonstrate that the disclosure in the specification meets the enablement requirements for patentability of the claimed invention.

The first, a report by PIALOUX et al. 2001, AIDS 15(10) 1239-1249 entitled "Lipopeptides induce cell-mediated anti-HIV immune responses in seronegative volunteers" is attached as Exhibit 1. At page 1240, first column, discloses the use of lipid modified peptides

containing CTL epitopes derived from HIV-1 NEF, GAG and ENV that also contain T-cell (and B-cell) epitopes in human vaccine trials. In particular, a lipopeptide containing the GAG 253 antigenic determinant is disclosed at Table 1.

The vaccine preparations including the GAG 253 lipopeptide were administered to human volunteers (See "Study design" at page 1240). The experimental data show that the lipopeptide vaccine elicits a cytotoxic T-cell response in humans.

Further, at page 1246, first column at (2), the authors state "All the responses were confirmed at least twice in each responder whereas in most previously published trials of HIV vaccination, most of the positive responses were detected only once and could not be confirmed subsequently."

Also, at page 1246, first column at (4) Pailoux et al. disclose that the vaccine compositions containing the claimed mixed micelles or micro-aggregates elicited polytypic responses in more than half (seven of 13) of the responders, whereas the prior art vaccines generally elicited monotypic responses.

Moreover, at page 1248, first column lines 1-4, the authors conclude that the disclosed vaccine preparation of mixed micelles or micro-aggregates "are capable of inducing reproducible polyepitopic, and strong CTL responses in at least half the responders associated with clear T cell proliferation and antibody responders."

The second, a report by GAHERY-SEGARD et al., 2000, *J. Virology* 74(4) 1694-1703 entitled "Multiepitopic B- and T-Cell Responses Indiuced in Humans by a Human Immunodeficiency Virus Type I Lipopeptide Vaccine" is attached as Exhibit 2. This publication discloses data from a phase I clinical trial using the claimed mixed micelles or micro-aggregates against HIV, which contain the GAG 253 antigenic determinant, (see page 1695, Table 1, line 5) and the use of the TT 830-843 epitope (page 1701, second column, last paragraph). The experimental data also demonstrate an efficient cytotoxic T-cell response elicited against HIV-1 in humans (see for example Tables 4 and 5 at page 1699) with strong and multiepitopic B- and T-cell responses. (See abstract).

At page 4 of the Office Action the Examiner cites Vergis et al. to support the contention that HIV infection results in depletion of CD4+ T-lymphocytes and goes on to state that the disclosure does not present any data indicating that a proliferative response in CD4+ T cells would be of benefit in treating an individual infected with HIV. The Examiner goes on to cite Kornbluth et al. for the teaching that activation of APCs leads to increased CD4+ activation, leading to increased HIV replication in these cells.

Applicants respond that the Examiner has not demonstrated a sufficient reason to believe that the combined effects of the multi-epitopic immune responses of the vaccines of the invention will not prove effective against HIV. In particular, the Examiner has presented no

evidence of lack of effectiveness of the vaccine. Indeed, the inventors and the French government *(Agence Nationale de Recherches sur le SIDA)* were sufficiently confident of its effectiveness to sponsor the human trials reported in the publications attached as Exhibits 1 & 2.

Applicants believe that the attached exhibits provide ample evidence to convince one of skill in the medical arts that the claimed mixed micelle or micro-aggregates are functional in inducing an immune response as claimed and therefore that the claims are fully enabled without undue experimentation. Therefore, Applicants assert that the rejections of claims 13, 14, 16 and 20 for lack of enablement is unsupported by the facts and should be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 1-7, 9-10, 13-17 and 19-24: At page 5 of the Office Action the Examiner rejected claims 1-7, 9-10, 13-17, 19-24 as obvious over Stuhler et al. and Sastry et al. and Sugimoto et al. The Stuhler et al. reference is cited for the teachings of the critical linkage of epitopes for helper and CTLs on the surface of one APC, using HIV-gag as the CTL epitope and various helper epitopes including KLH, pigeon cytochrome c, and TT. The Examiner acknowledged that Stuhler et al. do not teach the use of HA as a helper epitope. Sugimoto et al. is cited for the teaching that the HA epitope elicits a similar immune response to KLH.

At the last paragraph on page 5, the Examiner states that "Stuhler et al. does not teach conjugating the CTL epitope to the helper epitope by palmitic acid residues in a micelle

composition." Further, the Examiner states at page 6, first paragraph that one of ordinary skill "would have been motivated to combine the method of stimulating helper, CTl and APC immune responses by combining the helper and CTL epitopes taught by Stuhler et al. in the micelle composition taught by Sastry et al. because the linked micelles epitopes would contact the same APC at the same time and the epitopes would not require a carrier molecule."

It is respectfully submitted that the claimed subject matter rejected by the Examiner does not correspond to the claimed invention. Nowhere in the present specification and claims is there any disclosure of or claim to conjugating the CTL epitope to the helper epitope by palmitic acid residues in a micelle composition, nor is there any teaching of such "linked micelle epitopes." The invention provides mixed micelles or micro-aggregates that include at least one CTL epitope and at least one helper epitope. Nowhere is there any disclosure or claim of a CTL epitope conjugated to a helper epitope.

Therefore, the Examiner's rejection of claims 1-7, 9-10, 13-17, 19-24 as obvious over Stuhler et al. and Sastry et al. and Sugimoto et al. is misplaced and should be withdrawn.

Applicants further point out that one of ordinary skill in the art at the time the invention was made would have had no motivation to combine the teachings of the Stuhler et al. and the Sastry et al. references.

When reading the Stuhler et al. reference one of ordinary skill is given no indication of any preferred mode of presentation of the antigenic determinants to the APCs. At most, Stuhler et al. teach that CTL and helper T antigens are requires to elicit a cytotoxic T-cell response. See page 622, second column, last sentence before MATERIALS AND METHODS. This sentence recites: "Coordination of T cellular interactions appear to be organiozed by linkage of antigens on the APC."

Further, Sastry et al. teach the induction of a cytolytic response against HIV without the simultaneous induction of an antiviral antibody response (see page 700 first column, beginning of the first paragraph). The main goal of Sastry et al. was to dissociate the induction of a cytolytic response from the induction of an antiviral antibody response (as disclosed at page 704, firat col). Sastry et al. approached this problem by using polymers wherein the peptides are linked to each other in a single peptide polymer.

Therefore, one of ordinary skill in the art would have been dissuaded from using peptide preparations containing mixtures of peptides. Rather, the skilled artisan would have been led to a preparation comprising a CTL conjugated to a helper epitope, as sugested by the Examiner.

For all the above reasons, Applicants maintain that the claimed invention is not obvious over Stuhler et al. and Sastry et al. and Sugimoto et al. and should be withdrawn.

Claim 11: At page 6 of the Office Action, the Examiner rejected claim 11 as obvious over Stuhler et al. and Sastry et al. and Sugimoto et al. and further in view of Kramer et al. The Examiner points to the Kramer reference for the disclosure of the GAG 253 peptide as an immunogenic sequence and its use in detection assays and pharmaceutical compositions.

As explained above, the combination of Stuhler et al. and Sastry et al. and Sugimoto et al. do not lead to the claimed invention of mixed micelles or micro-aggregates comprising micelles or micro-aggregates that each contain a first lipopeptide with a CTL antigenic determinant and at least one lipid unit and a second lipopeptide with a helper T-antigenic determinant and at least one lipid unit. Addition of the teachings of Kramer et al. concerning the GAG 253 peptide sequence fails to repair this defect.

Therefore, for all the above reasons, Applicants maintain that the invention of claim 11 is not obvious over the four way combination of Stuhler et al. and Sastry et al. and Sugimoto et al and Kramer et al., and should be withdrawn.

Claim 18: At page 7 of the Office Action the Examiner rejected claim 18 as obvious over Stuhler et al. and Sastry et al. and Sugimoto et al. and Kramer et al. and further in view of Shapiro et al. The latter reference is cited for the teaching of the use of two-dimensional magnetic

resonance to aid in analyzing the conformation of micelle/peptide-receptor interactions. According to the Examiner, one of ordinary skill "would have been motivated to utilize two-dimensional NMR to facilitate the interaction between the targeted APC and the micelle."

Applicants have amended claim 18 to recite "A method according to claim 17 wherein the dispersing of the lipopeptides dissolved in acetic acid is confirmed by a two-dimensional nuclear magnetic resonance method."

Again, as explained above, the combination of Stuhler et al. and Sastry et al. and Sugimoto et al. does not lead to the claimed invention of mixed micelles or micro-aggregates for inducing an immune response. The addition of the teachings of Shapiro et al. concerning the use of two-dimensional NMR fails to repair this defect.

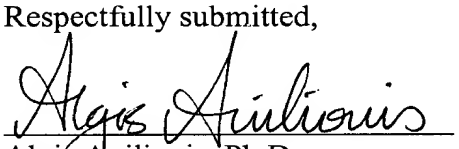
Therefore, for all the above reasons, Applicants maintain that the invention of claim 18 is not obvious over the five-way combination of Stuhler et al. and Sastry et al. and Sugimoto et al. and Kramer et al. and Shapiro et al., and should be withdrawn.

Changes to the claims are supported by the claims as originally filed and throughout the specification. No new matter has been added by the above amendments.

Attorney for Applicants respectfully requests entry of the above amendments and reconsideration of the rejections of the pending claims on the basis of the remarks rpesented herein.

If the Examiner has any questions arising from this amendment that could be addressed by the attorney of record, the Examiner is respectfully invited to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification

Please delete the paragraph overlapping from page 9, line 10 to page 10, line 8 and replace with the following:

-- The quality of dissolution, i.e. the effective dispersion at the molecular level of each lipopeptide before the preparation of the mixture, is [controlled] confirmed by the two-dimensional nuclear magnetic resonance method (2DNMR). The resolution of the signal obtained during homonuclear experiments in two dimensions in a 600 MHz field confirms the complete dispersion, at the molecular level, of the lipopeptides in solution. The clarity of the mixture is not a sufficient criterion: in particular, the taking up of the lipopeptides by DMSO or a DMSO/water mixture does not lead, in most cases, to a sufficient dispersion state, which explains the ineffectiveness of the mixture studied by VITIELLO et al. (1995, cited above). Dissolution by acetic acid/water mixtures which are more dilute in acetic acid also does not lead in all cases to the preparation of a mixture of mixed micro-aggregates or micelles containing a statistical proportion of each constituent of the mixture at the microvolume level. In these two cases, even in the presence of an apparently clear mixture, the sterilizing filtration over a 0.22 μm membrane is either impossible, or irregular, with filtration yields which differ according to the constituents, which indicates that at the scale of a particle of this size, the representation of

each constituent of the mixture has not been achieved. This micro-heterogeneity compromises the immunogenicity of the mixture, since it comprises the simultaneous capture and presentation of all constituents by a single antigen-presenting cell (APC), in the case of CTL and HTL antigenic determinants present on separate lipopeptides. --

In the claims

Please amend claims 1-6, 9-11, 13-16, 18-20 and 22 and add new claim 25 as follows: (For the Examiner's convenience all the pending claims, 1-7, 9-11 and 13-25 are reproduced in full below):

1. (Twice amended) A composition [of mixed micelles or micro-aggregates] for inducing an immune response, comprising micelles or micro-aggregates wherein each micelle or micro-aggregate comprises:

a first lipopeptide comprising at least one CTL antigenic determinant and at least one lipid unit, and

a second lipopeptide comprising at least one helper T antigenic determinant and at least one lipid unit.

2. (Twice amended) A composition according to claim 1 wherein the first and second lipopeptides each [independently] comprise one or more C_4-C_{18} lipid units.

3. (Twice amended) A composition according to Claim 1 wherein the first and second lipopeptides each [independently] comprise one or two C_4-C_{18} lipid chains linked by a covalent bond to one or two amino acids of the respective lipopeptide [peptide part].

4. (Twice amended) A composition according to Claim 1 wherein the lipid units of the lipopeptides [are composed of] each comprise two palmitic acid chains linked to [the NH_2 groups of] a lysine through an NH_2 group of said lysine.

5. (Twice amended) A composition according Claim 1 wherein the lipid units of [the lipopeptides independently] each lipopeptide comprises one or more of: a residue of palmitic acid, 2-aminohexadecanoic acid, oleic acid, linoleic acid, linolenic acid, pimelautide, trimexautide, or a derivative of cholesterol.

6. (Twice amended) A composition according to Claim 1 wherein the non-lipid part of the each of the first and second lipopeptides[, comprising the antigenic determinant,] comprises between 10 and 100 amino acids.

7. A composition according to Claim 1 wherein the helper T antigenic determinant is a multivalent antigenic determinant.

9. (Twice amended) A composition according to Claim 1 wherein the helper T antigenic determinant [is] comprises the antigenic determinant of hemagglutinin or the PADRE antigenic determinant.

10. (Twice amended) A composition according to Claim 1 wherein the lipopeptides comprise at least one CTL antigenic determinant selected from the group consisting of a specific protein of melanoma, a protein from HIV, a protein from HBV, a protein from papillomavirus, [or] protein p53[, or] and a specific protein of *Plasmodium falciparum*.

11. (Twice amended) A composition according to Claim 1 wherein said micelles or micro-aggregates comprise one or more of the following lipopeptides:

GAG 17 EKIRLRPGGKKKYKLKHIVK(Pam)-NH_2 (SEQ ID No: 31)

GAG 253 NPPIPVGEIYKRWIILGLNKIVRMYSPTSILDK(Pam)-NH_2 (SEQ ID No: 6)

POL 325 AIFQSSMTKILEPFRKQNPDIVIYQYMDDLYK(Pam)-NH_2 (SEQ ID No: 32)

NEF 66 VGFPVTPQVPLRPMTYKAAVDLSHFLKEKGGLK(Pam)-NH_2 (SEQ ID No: 2)

NEF 116 HTQGYFPDWQNYTPGPGVRYPLTFGWLYKLK(Pam)-NH_2 (SEQ ID No: 33)

TT Ac-QYIKANSKFIGITELKKK(Pam)-NH_2 (SEQ ID No: 30).

13. (Twice amended) A method for the production of [a drug or] a vaccine for inducing [a specific] an immune response comprising micelles or aggregates according to Claim 1.

14. (Twice mended) A method according to Claim 13, [wherein] said [specific] immune response [is] being induced against HIV, HBV, papilloma virus, p53, melanoma, or [malaria induced by] *Plasmodium falciparum.*

15. (Twice amended) A pharmaceutical composition comprising a pharmacologically effective dose of micelles or micro-aggregates according to Claim 1 and a pharmaceutically compatible vehicle[s].

16. (Twice amended) A [drug or] vaccine comprising micelles or micro-aggregates according to Claim 1 and a physiologically acceptable vehicle.

17. A method for producing micelles or micro-aggregates according to Claim 1, comprising the following steps:

- dispersing each of the constituent lipopeptides in a solution of concentrated acetic acid of about 80% concentration then
- mixing the solutions thus obtained.

18. (Twice amended) A method according to Claim 17 wherein the dispersing of the lipopeptides dissolved in acetic acid is [controlled] confirmed by a two-dimensional nuclear magnetic resonance method.

19. (Twice amended) A method for inducing an immune response against a particular antigen in an individual comprising [at least the administration of] administering micelles or micro-aggregates according to Claim 1 to [an] the individual [for whom such a response is desired].

20. (Twice amended) A method of [immunization] immunizing an individual against a pathogenic agent comprising the administration of micelles or micro-aggregates according to Claim 1 to [an] the individual [for whom such an immunization is sought].

21. A method according to Claim 19, wherein the pathogenic agent is HIV, HBV, papillomavirus, melanoma or *plasmodium falciparum*, and wherein the antigen is an antigen of one of said pathogenic agents, or p53.

22. (Twice amended) A composition according to Claim 1, wherein the at least one lipid unit in the second lipopeptide is different from [the at least one] any lipid unit in the first lipopeptide.

23. A composition according to Claim 6, wherein the non-lipid part of the lipopeptides, comprising the antigenic determinants, comprises between 10 and 50 amino acids.

25. A method according to Claim 20, wherein the pathogenic agent is HIV, HBV, papillomavirus, melanoma, or *Plasmodium falciparum*, and wherein the antigen is an antigen of one of said pathogenic agents, or p53.

25. (New) A composition according to Claim 8, wherein the helper T antigenic determinant is the peptide 830-843 of the tetanus toxin with the following sequence:

QYIKANSKFIGITE (SEQ ID No: 1).